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DIFFERENCES IN THE PERIODONTAL MICROBIOME OF SUCCESSFULLY TREATED AND PERSISTENT AGGRESSIVE PERIODONTITIS

Running title: Microbiome in aggressive periodontitis

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ABSTRACT

Aims: The primary aim of this investigation was to analyse the periodontal microbiome in patients with aggressive periodontitis (AgP) following treatment.

Methods: 66 AgP patients were recalled on average 7 years after completion of active periodontal treatment and had subgingival plaque samples collected and processed for 16S rRNA gene sequencing analyses.

Results: 52 out of 66 participants showed persistent periodontal disease, while 13 participants were considered as 'successfully-treated AgP' (no probing pocket depths >4mm) and 1 was fully edentulous. Genera associated with persistent generalised disease included *Actinomyces*, *Alloprevotella*, *Capnocytophaga*, *Filifactor*, *Fretibacterium*, *Fusobacterium*, *Leptotrichia*, *Mogibacterium*, *Saccharibacteria [G-1]*, *Selenomonas* and *Treponema*. 'Successfully treated' patients harboured higher proportions of *Haemophilus*, *Rothia*, and *Lautropia* and of *Corynebacterium*, *Streptococcus*, and *Peptidiphaga* genera. Overall, patients with persistent Generalised AgP (GAgP) revealed higher alpha diversity compared to persistent Localised AgP

(LAgP) and stable patients ($p<0.001$). Beta diversity analyses revealed significant differences only between stable and persistent GAgP groups ($p=0.004$).

Conclusion: Patients with persistent AgP showed a more dysbiotic subgingival biofilm than those who have been successfully treated. It remains to be established whether such differences were predisposing to disease activity or were a result of a dysbiotic change associated with disease recurrence in the presence of sub-standard supportive periodontal therapy or other patient-related factors.

Clinical Relevance

Scientific rationale for the study: The level of dysbiosis associated with aggressive periodontitis disease recurrence is currently unknown.

Principle findings: Higher microbial diversity was detected in active vs. stable aggressive periodontitis cases. Elevated differences at both genus and species levels were detected, mainly between persistent GAgP and successfully treated group. Overweight and irregular interdental brushing are associated with dysbiosis.

Practical implications: Increased dysbiosis, oral hygiene and obesity should be controlled to avoid dysbiosis-associated periodontal disease recurrence.

INTRODUCTION

Aggressive Periodontitis (AgP) was introduced as a disease entity by the 1999 World Workshop classification (Lang et al. 1999) to define a specific condition characterised by rapid disease progression in otherwise systemically healthy patients with a positive family history of periodontal disease. Among secondary features, elevated proportions of bacteria such as *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* were considered typical in some populations. In particular, an association between *A. actinomycetemcomitans* and AgP had been suggested in a large study in the United States using culture and immunofluorescence serotyping (Zambon et al. 1983) and confirmed by studies observing treatment response (Mandell et al. 1987) and antibody response (Ebersole et al. 1994, Albandar et al. 2001). The JP2 clone of *A. actinomycetemcomitans* has been consistently associated with onset and progression of periodontal attachment loss in children and adolescents in North and West Africa (Haubek et al. 2008, Höglund Åberg et al. 2014) as well as in the United States (Fine et al. 2007). However, the great majority of comparative studies suggest that the microbial differences between AgP and Chronic Periodontitis are so limited (Lafaurie et al. 2007, Riep et al. 2009, Nibali et al. 2012) that they are no longer considered different disease entities (Tonetti et al. 2018); a conclusion supported also by evidence from other pathogenicity aspects (Tonetti et al. 2018).

The introduction of molecular techniques suggested a role for non-cultivable microbes as *Filifactor alocis*, *Centipeda* genus, *Mitsuokella* sp., *Selenomonas* genus, *Actinobacter baumannii* in the pathogenesis of periodontitis (Schlafer et al. 2010, Goncalves et al. 2012, Schulz et al. 2019) depicting a more variegated picture than previously thought, in line with the concept of polymicrobial synergy and dysbiosis (Hajshengallis & Lamont 2014). However, the role of these putative periodontopathogenic bacteria has not been fully elucidated yet, and little is known about their potential to instigate periodontal disease recurrence during maintenance therapy.

The primary aim of this investigation was to analyse the periodontal microbiome in previously-treated patients with AgP and to associate it with the presence of active periodontal disease or health. We also investigated the relationship between the AgP microbiome composition and association between regular supportive periodontal therapy, tooth loss and patient-related factors, such as oral hygiene, smoking, and body mass index.

MATERIALS AND METHODS

Patient population

The study design and population have been previously described (Dopico et al. 2016). Briefly, patients seen in a dedicated AgP clinic were contacted and asked to participate in the study. Eighty-two patients were contactable of the original 330 with AgP, and 66 agreed to participate, gave informed consent and completed the study visit to the Eastman Dental Hospital (EDH), in London. The study was conducted in line with the principles outlined in the Declaration of Helsinki (2008) on experimentation involving human participants. Ethics approval for the conduct of the study was granted by the NRES Committee London – Queen Square (ref 13/LO/0874).

Inclusion criteria were: i) previous diagnosis of AgP – in the dedicated AgP clinic at the EDH between 1997 and 2011, and ii) age between 16–65 at recruitment. Exclusion criteria were: i) known infectious diseases such as Tuberculosis, HIV or hepatitis C, ii) comorbidities that would make it unsafe for the patient to travel or to undergo periodontal assessment, and iii) pregnancy. Following initial diagnosis, patients had received active periodontal therapy (APT) consisting of oral hygiene instructions, non-surgical treatment, adjunctive antibiotics and/or surgical treatment according to clinical needs (Table 1). Following completion of APT patients had a full periodontal assessment and were then discharged to their general dental practitioner for supportive periodontal therapy (SPT).

At the study visit, following consent, patients were interviewed to obtain self-reported medical, dental and smoking histories. Participants' height and weight were measured and body mass index (BMI) calculated. Patients were classified as 'Normal weight' if their BMI was $<25 \text{ kg/m}^2$, 'Overweight' if their BMI was $25\text{--}29 \text{ kg/m}^2$ and 'Obese' if their BMI was $\geq 30 \text{ kg/m}^2$. Clinical data were collected at 6 sites per tooth by one calibrated examiner (JD) including Full-Mouth dichotomous Plaque Score (FMPS) (Guerrero et al., 2005), Gingival Index (Löe, 1967), Tooth Mobility (Laster et al., 1975), Tooth loss, Furcation Involvement (Hamp et al., 1975), Probing Pocket Depth (PPD), Gingival Recession (Distance from the Cemento-Enamel Junction to the Free Gingival Margin) and Full-Mouth dichotomous Bleeding on Probing Score (FMBS, percentage of bleeding surfaces upon probing). Clinical Attachment Level (CAL) was measured as PPD plus recession.

Clinical diagnosis

At the study visit, the following diagnoses could be assigned:

- i) Persistent AgP: in case of interproximal PPD and CAL ≥ 5 mm on at least two permanent teeth. Subdivided into either: LAgP (at least one affected first molar or incisor, and no more than three teeth other than first molars or incisors), or GAgP (affecting at least three permanent teeth other than first molars and incisors).
- ii) Successfully treated AgP: no teeth with PPD ≥ 5 mm.

We defined 'quality of SPT' during the follow-up period based on hospital records and patient interview as: i) no maintenance, ii) sub-optimal, or iii) optimal. Optimal maintenance was defined as those programs with at least 3 recall visits/year including oral hygiene advice and support, supra- and sub-gingival debridement and charting. Any SPT program which did not fulfil the criteria for being considered optimal, was considered suboptimal. 'No maintenance' was defined for patients who received no maintenance care at all after active periodontal therapy (Dopico et al. 2016).

Subgingival plaque sampling

For every patient, samples from each quadrant were taken from the disto-buccal surfaces of all first molars with sterile curettes and pooled together. In case of absence of any of these teeth, the neighbouring teeth were chosen. The supragingival portion of the root surface of the site was carefully cleaned with a curette. With the area isolated from saliva (gentle air spray or cotton rolls) a sterile curette was inserted to the bottom of the pocket. After a single stroke each microbiological sample was extracted from the pocket and then immediately placed into 1 mL of reduced transport fluid (RTF) and stored at -80°C for future 16S rRNA gene sequencing analysis.

Microbiological analysis

A site-based sub-analysis comparison of the periodontal and peri-implant microbiomes of 18 patients included in this study, reporting the microbiological techniques employed, has previously been published (Sousa et al. 2017). Briefly, plaque samples were processed for DNA extraction by bead and beating technique and then for sequencing analysis using the Illumina platform (MiSeq

Desktop Sequencer; Reagent Kit v2; Illumina Inc., San Diego, CA, US). The PCR mixtures were prepared as described (Sousa et al., 2017). The products from the PCR were then quantified, purified, and combined in equimolar ratios, to create a DNA library for 16S rRNA sequencing. The 16S rRNA gene fragments were sequenced by NGS (MiSeq Desktop Sequencer; Reagent Kit v2; Illumina Inc., San Diego, CA, US). DNA was extracted (Griffiths et al., 2000) followed by individual PCR reactions for each sample in order to “amplify the V5-V7 hyper-variable region with barcoded primers 785F (F 5’- GGATTAGATACCCBRGTAGTC-3’) and 1175R (R 5’- ACGTCRTCCCCDCCTTCCTC-3’).

Statistical and bioinformatic analyses

A convenience sample was chosen for this study, based on the number of patients able to attend a re-evaluation appointment. Anonymised clinical data from all patients who took part in the study were entered into a database, proofread for entry errors and analysed by a statistical package (IBM SPSS (25.0)). Continuous variables are reported as means, percentages, and standard deviations. The patient was the unit of analysis. Patients were grouped by disease activity (diagnosis of ‘persistent GAgP’, ‘persistent LAgP’ or ‘successfully treated AgP’). Further exploratory analyses were carried out by grouping patients by tooth loss during the follow-up period (‘at least 1 tooth lost’ vs. ‘none’), smoking status (current or not), BMI (‘normal weight’ vs. ‘overweight or obese’) and interdental brushing (‘daily’ or ‘not’).

Sequencing data were processed using QIIME 2 (Version 2018.11, <https://qiime2.org/>) (Bolyen *et al.*, 2019). Raw sequences belonging to 65 samples were demultiplexed with q2-demux then denoised using DADA2 plugin with default parameters to create Amplicon Sequence Variants (ASVs) (Callahan et al., 2017, 2016). Two samples (TM1 and T63) were removed from the resulting feature-table due to having extremely low number of sequences (< 232 sequences). Alpha diversity metrics were calculated on the unrarefied feature-table using native q2-diversity plugin, and included Number of ASVs, Shannon Index and Faith’s Phylogenetic Diversity. For beta diversity, the feature-table was rarefied at 2477 sequences and used to calculate Aitchison (compositional) and weighted UniFrac (quantitative, accounts for phylogenetic information) distances (Aitchison *et al.*, 2000; Lozupone *et al.*, 2011). These distance matrices were then fed to Principal coordinate analysis (PCoA) to investigate clustering patterns based on the metadata groupings. A taxonomic classifier optimised for V5-V7 region of the 16S rRNA gene from the

extended human oral microbiome database (HOMD extended, <https://tinyurl.com/HOMD-Extended>) was trained using q2-feature-classifier, and taxonomy was assigned using this classifier to ASVs at >85% confidence (Bokulich et al., 2018). With the unrarefied feature-table, q2-gneiss plugin was used to determine taxonomic associations between healthy and disease samples at genus and species level (Morton *et al.*, 2017). For this, a hierarchical tree was generated based on co-occurrence correlations among taxonomic units, followed by partitioning using simplicial linear regression accommodating available clinical data. From the top three balances created, y0 and y2 had the highest coverage of the taxonomic tree, and so were used to identify taxonomic associations. Furthermore, the unrarefied feature-table was transferred to QIIME 1 platform to generate group-wise (health/disease, daily interdental brushing, body weight) taxonomic summaries using a *summarize taxa through plots.py* function (Caporaso *et al.*, 2010). The data resulting from the analyses mentioned above was exported from QIIME 2 environment, and respective plots were generated in R v.3.5.0 using ggplot2 package v.3.1.0 (Wickham, 2016; R Core Team, 2018).

The statistical significance of the alpha diversity metrics, and the y0 and y2 balances among the persistent and stable periodontitis groups were tested using Mann-Whitney U test, and $p \leq 0.05$ was accepted as significant. For microbial profiles, statistical significance at phylum, genus and species level between GAgP, LAgP and successfully treated groups were tested using ALDEx2 package in R. Wilcoxon signed-rank test combined with Benjamini-Hochberg correction was reported, and $p < 0.05$ was accepted as significant (Fernandes *et al.*, 2013, 2014). The clustering of these groups in beta diversity analyses was tested using group-wise PERMANOVA (999 permutations) and $p < 0.05$ was accepted as significant.

RESULTS

Demographic characteristics of the 66 patients included in the study have been previously reported (Dopico et al. 2016). Relevant current study population characteristics are included in Table 1. Forty-nine patients had been diagnosed with GAgP and 17 as LAgP at first examination. Based on the current classification (Tonetti, Greenwell & Kornman, 2018, Papapanou et al. 2018), 42 patients would have been diagnosed as generalised stage III grade C, 7 patients as generalised stage IV grade C and 17 as molar-incisor pattern stage III grade C at first examination. At the study visit, 2 patients reported diagnosis of diabetes mellitus, 5 reported cardiovascular disease

and 2 reported rheumatoid arthritis – all of these were new diagnoses during the follow-up period. The majority of patients (58%) were Caucasians. The mean time in SPT was 6.97 years (95% CI 6.25–7.69, range 2–11 years). At the study visit, a total of 8 participants (12%) were current smokers. A large proportion of participants was overweight ($n=20$, 31%) or obese ($n=18$, 28%). Twenty-four percent of patients underwent what we defined as ‘optimal’ maintenance during the study follow-up. Half of the patients ($n=33$) lost at least 1 tooth during the follow-up period. Thirteen participants (20%) were diagnosed as ‘successfully treated’, 52 (79%) were diagnosed as having persistent AgP (GAgP: $n=44$; LAgP $n=8$), while one was edentulous (Table 1). The mean PPD of all sampled sites was 4.06mm (SD ± 0.86 mm) and the mean PPD in the deepest sampled pocket per patient was 5.26 mm (SD ± 1.75 mm).

Community Characterisation

The alpha diversity measures of richness, evenness, and phylogenetic diversity indicated clear differences appearing between the three groups. Overall, persistent GAgP samples revealed higher microbial richness and diversity compared to the other two groups (Figure 1). The mean number of ASVs in the GAgP group was 205 ± 101 , in LAgP 141 ± 58 , and in successfully treated group was 94 ± 41 (Figure 1a).

Phylum level analysis

Samples taken from patients with ‘persistent AgP’ (localised and generalised) had higher relative abundances of *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Spirochaetes*, *Fusobacteria*, *Synergistetes*, and *Saccharibacteria* phyla compared with patients with stable periodontitis (see supplemental Figure 1). Moreover, the *Chloroflexi* and *Gracilibacteria* phyla appeared in higher proportions in the GAgP group sample in comparison to the LAgP and ‘successfully treated’ groups. The distribution of phyla appears to share similarities in the richness between samples across disease groups, with *Actinobacteria* and *Firmicutes* as dominant phyla. However, a variety of gram-negative phyla, such as *Bacteroidetes* and *Fusobacteria* appear with higher proportions in the ‘persistent AgP’ group. The obese group exhibited increases in *Fusobacteria* and *Spirochaetes* phyla when compared to the normal-weight group.

Genus and species level analysis

Two balances, y0 and y2, with the highest coverage of the taxonomic tree, showed significant differences between the GAgP and successfully treated groups. The underlying taxa from these balances at genus and species level were extracted (Supplementary Figure 2). At genus level, GAgP patients had higher proportions of Gram-negative obligate anaerobes (putative periodontal pathogens), and of *Actinomyces*, *Alloprevotella*, *Capnocytophaga*, *Filifactor*, *Fretibacterium*, *Fusobacterium*, *Leptotrichia*, *Mogibacterium*, *Saccharibacteria* [G-1], *Selenomonas*, *Porphyromonas* and *Treponema* genera (Figure 2), whereas LAgP patients presented higher proportions of *Rothia*, *Neisseria*, *Dialister*, and *Saccharibacteria* (TM7) [G-5]. In the ‘successfully treated’ group there were higher proportions of *Haemophilus*, *Rothia*, and *Lautropia* (Figure 3), and of *Corynebacterium*, *Streptococcus*, and *Peptidiphaga* genera (see supplemental material 2 and 3).

At species level, persistent periodontitis was associated with periodontal bacteria including *Capnocytophaga granulosa* [HMT 325], *Actinomyces oris* [HMT 893], *Solobacterium moorei* [HMT 678], *Actinomyces naeslundii* [HMT 176], *Actinomyces odontolyticus* [HMT 701], *Fusobacterium nucleatum*, *Filifactor alocis* [HMT 539], *Treponema socranskii* [HMT 769], *Treponema denticola* [HMT 584], *Leptotrichia wadei* [HMT 222], *Treponema* sp. [HMT 237], *Selenomonas sputigena* [HMT 151] (Figure 3a). In comparison to sites from patients with persistent GAgP, sites from successfully treated patients exhibited higher levels of *Actinomyces graevenitzii* [HMT 866], *Haemophilus parainfluenzae* [HMT 718], *Capnocytophaga sputigena* [HMT 775], *Rothia mucilaginosa* [HMT 681], *Lautropia mirabilis* [HMT 022] (Figure 3b).

Notably, ALDEx2 testing did not reveal any statistically significant association between the LAgP and stable periodontitis at genus and species level analysis. However, *Treponema* genus ($p = 0.013$) was found significantly higher abundance in the GAgP group, and *Rothia* ($p = 0.012$) and *Haemophilus* ($p = 0.032$) genera were in higher abundance in the successfully treated group. Similarly, at species level *Treponema* sp. [HMT 237] ($p = 0.044$) and *Treponema socranskii* [HMT 769] ($p = 0.038$) were significantly higher in the GAgP group, while *Granulicatella adiacens* [HMT 534] ($p = 0.040$) was significantly higher in the successfully treated group. In agreement with this, significant differences in Beta diversity were detected between successfully treated and GAgP groups ($p = 0.004$), but not between the LAgP and successfully treated groups ($p > 0.172$) (Figure 4).

Analysis of patient factors associated with diversity

Smoking: Higher proportions of *Corynebacterium*, *Gemella*, and *Bacteroidales* [G-2] genera were observed in the non-smoker group, whereas the smoker group exhibited higher genera *Actinomyces*, *Prevotella*, *Porphyromonas*, *Neisseria*, *Clostridiales*, and *Fretibacterium* (supplemental Figures 4 and 5). However, no statistically significant changes in alpha diversity were detected in smokers vs. non-smokers ($p = 0.381$).

Tooth loss: No significant differences in alpha diversity were observed when comparing patients who had lost teeth during the study follow-up to patients who had not lost teeth ($p = 0.320$).

BMI: Statistically significant difference ($p = 0.042$) in alpha diversity was observed comparing patients by BMI (overweight/obese vs. normal weight). Overweight/obese patients had higher proportions of *Actinomyces*, *Streptococcus*, *Selenomonas*, *Treponema*, *Leptotrichia*, *Veillonella*, *Capnocytophaga*, *Actinomycetaceae*, and *Clostridiales* (supplemental figures 4 and 5).

Interdental cleaning: Statistically significant difference ($p < 0.001$) in alpha diversity was observed between patients using daily interdental brushing in comparison to irregular interdental brushing. Patients using daily interdental brushing had higher proportions of *Actinomyces*, *Streptococcus*, *Corynebacterium*, *Rothia*, *Peptidiphaga*, and *Veillonella* (supplemental figures 4 and 5).

DISCUSSION

This study explored the subgingival microbiome of patients affected by AgP, following an average of 7 years of SPT. However, it is important to emphasise that the majority of patients had not received what we defined as ‘satisfactory SPT’ and 80% of them still had signs of ongoing or persistent disease, measured as multiple persisting sites with concomitant PPD and CAL ≥ 5 mm. Furthermore, 50% of patients lost at least one tooth during the SPT period, with an average tooth loss of 0.27 teeth/patient/year. We previously showed that having received surgical treatment, the performance of interproximal cleaning and shallower PPDs at start of SPT were found to be associated with lower annual tooth loss rates (Dopico et al. 2016). These results are in line with a recent study that reported an association between greater PPD reduction and frequent SPT recall visits in long-term maintenance periodontal patients (Müller Campanile et al. 2019). Diabetes

mellitus, obesity and smoking have also been reported as risk factors influencing the recurrence of number of sites with deep PPDs, positive BOP, and tooth loss (Kim et al. 2019; Müller Campanile et al. 2019).

This study shows, for the first time, clear differences in the subgingival microbiota between patients with persistent GAgP and patients considered ‘successfully treated’. Patients with ongoing disease had a more diverse microbiota, compatible with increased dysbiosis, in agreement with previous studies which seem to suggest that increased microbial diversity at subgingival level is associated with disease (Dabdoub et al. 2013, Sousa et al. 2017, Cui et al. 2019). More specifically, different bacterial species can be mapped according to the material and methods employed (Schulz et al. 2019) and these differences warrant further investigation.

Analysis by phyla revealed that *Chloroflexi* and *Gracilibacteria* were elevated in the persistent GAgP group when compared to the persistent LAgP and successfully treated groups, in agreement with the notion that Gram-negative bacteria are often elevated in periodontitis and particularly refractory periodontitis cases (Paster et al. 2001). The persistent GAgP-associated genera included *Actinomyces*, *Alloprevotella*, *Capnocytophaga*, *Filifactor*, *Fretibacterium*, *Fusobacterium*, *Leptotrichia*, *Mogibacterium*, *Saccharibacteria [G-1]*, *Selenomonas*, and *Treponema*, while the ‘successfully treated’ associated genera included *Haemophilus*, *Rothia*, and *Lautropia*.

Rothia, a Gram-positive, aerobic, rod-shaped and non-motile bacterial genus from the phylum *Actinobacteria* was the dominant genus. Several other studies using next generation sequencing have previously shown a consistent association between increased *Rothia* levels and periodontal health compared with chronic periodontitis (Abusleme et al. 2013, Camelo-Castillo et al. 2015b, Ikeda et al. 2019, Kirst et al. 2015). The latter study on 25 chronic periodontitis patients and 25 controls identified a disease-associated cluster driven by *Fusobacterium* and *Porphyromonas* and a health-associated cluster dominated by *Rothia* and *Streptococcus*, accompanied by alterations of the predicted functional capabilities of the periodontitis microbiome (Kirst et al. 2015). This finding is also in concordance with studies showing an increase in the relative abundance of *Rothia* following periodontal treatment, and suggesting that *Rothia* is associated with therapeutic success in chronic and aggressive periodontitis (Colombo et al. 2012, Junemann et al. 2012, Bizzarro et al. 2016, Han et al. 2017, Hagenfeld et al. 2018, Belstrøm et al. 2018). The latter study

in particular showed that successful periodontal treatment resulted in significantly higher relative abundance of *Streptococcus*, *Rothia* and *Actinomyces* in combination with a significant decrease in *Porphyromonas* and *Treponema* in subgingival plaque samples, demonstrating different shifts in relative abundance of health- and disease-associated bacteria following treatment (Belstrøm et al., 2018).

It was noteworthy that the *Saccharibacteria* (TM7) phylum was reduced in successfully treated participants compared with persistent AgP cases. This is in agreement with a microbiological analysis of chronic periodontitis patients, showing an increase in relative abundance of *Saccharibacteria* in periodontitis sites compared to healthy sites (Brinig et al. 2003). Another microbiological study estimated the increase in the relative abundance of *Saccharibacteria* to be threefold higher in smokers compared to non-smokers (Camelo-Castillo et al. 2015a).

At the bacterial species level, persistent AgP was associated with some recognised periodontopathogenic bacteria such as *Fusobacterium nucleatum*, *Filifactor alocis* and *Treponema denticola* as well as a series of potentially pathogenic bacteria (see Figure 3a), which is in agreement with a recent study on GAgP (Schulz et al. 2019). Another 16S study on 15 AgP and 15 healthy subjects identified species such as *F. alocis*, *Desulfobulbus* sp., *Fretibacterium* sp., *Porphyromonas gingivalis*, *Tannerella forsythia*, *Porphyromonas endodontalis*, *Peptostreptococcaceae* spp., *Parvimonas micra*, *Eubacterium nodatum* and *Eubacterium saphenum* as increased in AgP cases (Cui et al. 2019). In contrast, sites from successfully treated patients in the present study exhibited higher levels of *Actinomyces graevenitzii*, *Capnocytophaga sputigena*, *Rothia mucilaginosa*, *Haemophilus parainfluenzae* and *Lautropia mirabilis* (Figure 3b). Interestingly, two of these bacteria (*H. parainfluenzae* and *L. mirabilis*) were also the most abundant in healthy children when compared with AgP (Shaddox et al. 2012), while *C. sputigena* has previously been associated with good response to periodontal treatment (Colombo et al. 2012). *L. mirabilis* has also been found elevated in healthy subjects compared with chronic periodontitis patients in Japan (Ikeda et al. 2019). However, we should also point out that some bacteria normally associated with periodontal health, such as *A. oris*, *A. naeslundii* and *A. odontolyticus* (Abusleme et al. 2013) were also elevated in patients with persistent disease, which is difficult to explain, perhaps as a result of the study design and/or the analytic technique used.

Cigarette smoking has been associated with periodontal dysbiosis, regardless of periodontal phenotypes, possibly through alterations in the function of key periodontal pathogens (Hanioka et al. 2019). Although no statistically significant differences in alpha-diversity were detected in the present study according to smoking status, non-smokers had an overall higher proportion of Gram-positive bacteria such as *Corynebacterium* and *Gemella* genera, while smokers had a tendency to a higher proportion of Gram-negative genera such as *Prevotella*, *Porphyromonas*, *Neisseria*, and *Fretibacterium*. Another interesting finding in the present study is that the presence of the *Synergistetes* phylum (consisting of Gram-negative anaerobes) was increased in the smokers group compared to non-smokers. *Synergistetes* have been implicated in necrotising ulcerative gingivitis as well as in chronic periodontitis (Vartoukian et al. 2010), suggesting that smoking may predispose to a more pathogenic subgingival microbiota.

It is very important to note that daily interdental brushing was associated with a ‘healthier’ subgingival microbiota, characterised by reduced diversity and comprising of higher proportions of *Actinomyces*, *Streptococcus*, *Corynebacterium*, *Rothia*, *Peptidiphaga*, and *Veillonella*. This is not surprising, owing to the importance of oral hygiene in maintaining a healthy microbiota (Zhang et al., 2019). Interestingly, a large proportion of patients included in this study were overweight or obese. These conditions showed evidence of association with shifts in microbial communities subgingivally, measured as increased diversity. This is in agreement with a study showing an association between BMI and number of PPDs ≥ 4 mm and BOP in long-term maintenance patients (Müller Campanile et al. 2019). However, it is in contrast with an inverse correlation between BMI and diversity in subgingival plaque in diabetic patients (Tam et al. 2018). The association between obesity and subgingival microbiota mirrors the observation that obesity may predispose to gut dysbiosis, as observed in studies showing increased Firmicutes to Bacteroides ratio in obese individuals (Ley et al. 2010). Recently, the theory of an “obesogenic” microbiota was proposed, on the basis of experiments showing that bacteria from obese mice can be transferred to a germ-free recipient, resulting in increased adiposity as compared to transfer of a “lean” microbiota from lean mice (reviewed by Fak 2016). However, we have to acknowledge a relatively small sample in the present study and possible residual confounding, as socio-economics factors were not accounted for.

A strength of this study is the comparison of the microbiota in a relatively large group of AgP patients with and without disease progression over a long follow-up period (7 years). Drawbacks are absence of microbial data at baseline and different degrees and intensity of SPT regimes and the relative abundance of unclassified data in the results. Harvesting a single pooled subgingival sample per patient from standardised sites (rather than necessarily the deepest pockets) also represents a limitation, as it may have missed dysbiotic plaque and pathogenic taxa present in deeper pockets. This can be avoided by the use of whole genome sequencing, which is relatively expensive compared to the technique used in the current investigation (16S rRNA gene sequencing).

Overall, this study suggests that subgingival dysbiosis is associated with progression of periodontitis and that factors such as poor oral hygiene and obesity may have a negative impact on the composition of the subgingival microbiota.

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REFERENCES

- Abusleme, L., Dupuy, A.K., Dutzan, N., Silva, N., Burleson JA, Strausbaugh, L.D., Gamonal, J. & Diaz, P.I. (2013) The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. *ISME J.* 7, 1016-25.
- Aitchison, J., Barceló-Vidal, C., Martín-Fernández, J. A. & Pawlowsky-Glahn, V. (2000). Logratio Analysis and Compositional Distance. *Mathematical Geology* 32, 271–275.
- Albandar, J. M., DeNardin, A. M., Adesanya, M. R., Diehl, S. R., & Winn, D. M. (2001). Associations between serum antibody levels to periodontal pathogens and early-onset periodontitis. *Journal of Periodontology* 72, 1463-9.
- Belstrøm, D., Grande, M., Sembler-Møller, M., Kirkby, N., Cotton, S., Paster, B., & Holmstrup, P. (2018). Influence of periodontal treatment on subgingival and salivary microbiotas. *Journal of Periodontology* 89, 531-539.

- Bizzarro, S., Laine, M.L., Buijs, M.J., Brandt, B.W., Crielaard, W., Loos, B.G., Zaura, E. (2016) Microbial profiles at baseline and not the use of antibiotics determine the clinical outcome of the treatment of chronic periodontitis. *Sci Rep* 1, 6:20205.
- Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A. and Gregory Caporaso, J. (2018). Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* 6, 90.
- Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., Cope, E. K., Da Silva, R., Diener, C., Dorrestein, P. C., Douglas, G. M., Durall, D. M., Duvallet, C., Edwardson, C. F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J. M., Gibbons, S. M., Gibson, D. L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G. A., Janssen, S., Jarmusch, A. K., Jiang, L., Kaehler, B. D., Kang, K. Bin, Keefe, C. R., Keim, P., Kelley, S. T., Knights, D., Koester, I., Kosciulek, T., Kreps, J., Langille, M. G. I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B. D., McDonald, D., McIver, L. J., Melnik, A. V., Metcalf, J. L., Morgan, S. C., Morton, J. T., Naimey, A. T., Navas-Molina, J. A., Nothias, L. F., Orchanian, S. B., Pearson, T., Peoples, S. L., Petras, D., Preuss, M. L., Priesse, E., Rasmussen, L. B., Rivers, A., Robeson, M. S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S. J., Spear, J. R., Swafford, A. D., Thompson, L. R., Torres, P. J., Trinh, P., Tripathi, A., Turnbaugh, P. J., Ul-Hasan, S., van der Hooft, J. J. J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K. C., Williamson, C. H. D., Willis, A. D., Xu, Z. Z., Zaneveld, J. R., Zhang, Y., Zhu, Q., Knight, R. & Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* 37, 852–857.
- Brinig, M.M., Lepp, P.W., Ouverney, C.C., Armitage, G.C., Relman, D.A. (2003). Prevalence of Bacteria of Division TM7 in Human Subgingival Plaque and Their Association with Disease. *Applied Environmental Microbiology* 69, 1687–1694.
- Callahan, B. J., McMurdie, P. J. & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME Journal* 11, 2639–2643.
- Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A. & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* 13, 581–583.
- Camelo-Castillo, A., Novoa, L., Balsa-Castro, C., Blanco, J., Mira, A., Tomás, I. (2015b) Relationship between periodontitis-associated subgingival microbiota and clinical inflammation by 16S pyrosequencing. *Journal of Clinical Periodontology* 42, 1074-82.

- Camelo-Castillo, A.J., Mira, A., Pico, A., Nibali, L., Henderson, B., Donos, N., & Tomás, I. (2015a). Subgingival microbiota in health compared to periodontitis and the influence of smoking. *Frontiers Microbiology* 6.
- Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J. & Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7, 335–336.
- Colombo, A.P.V., Bennet, S., Cotton, S.L., Goodson, J.M., Kent, R., Haffajee, A.D., Socransky, S.S., Hasturk, H., Van Dyke, T.E., Dewhirst, F.E., & Paster, B.J. (2012). Impact of periodontal therapy on the subgingival microbiota of severe periodontitis: comparison between good responders and individuals with refractory periodontitis using the human oral microbe identification microarray. *Journal of Periodontology* 83, 1279–1287.
- Cui, Liu, Xiao, Chu, & Ouyang. (2019). Subgingival microbiome in Chinese patients with generalized aggressive periodontitis compared to healthy controls. *Archives of Oral Biology* 101, 92-99.
- Dabdoub, S.M., Tsigarida, A.A., & Kumar, P.S. (2013). Patient-specific analysis of periodontal and peri-implant microbiomes. *Journal of Dental Research* 92, 168S–75S.
- Dopico, J., Nibali, L., & Donos, N. (2016). Disease progression in aggressive periodontitis patients. A Retrospective Study. *Journal of Clinical Periodontology* 43, 531-7.
- Ebersole, J. L., & Cappelli, D. (1994) Gingival crevicular fluid antibody to *Actinobacillus actinomycetemcomitans* in periodontal disease. *Oral Microbiol Immunol* 9, 335-44.
- Fak, F. (2016) The gut microbiota: a predisposing factor in obesity, diabetes and atherosclerosis. In: *The Human Microbiota and Chronic Disease: Dysbiosis as a Cause of Human Pathology*. L Nibali & B Henderson. John Wiley & Sons, Inc.
- Fine, D. H., Markowitz, K., Furgang, D., Fairlie, K., Ferrandiz, J., Nasri, C., McKiernan, M., & Gunsolley, J. (2007). *Aggregatibacter actinomycetemcomitans* and its relationship to initiation of localized aggressive periodontitis: longitudinal cohort study of initially healthy adolescents. *Journal of Clinical Microbiology* 45, 3859-69.
- Fernandes, A. D., Macklaim, J. M., Linn, T. G., Reid, G. and Gloor, G. B. (2013). ANOVA-Like Differential Expression (ALDEx) Analysis for Mixed Population RNA-Seq. *PLoS ONE*, 8(7).
- Fernandes, A. D., Reid, J. N. S., Macklaim, J. M., McMurrough, T. A., Edgell, D. R. and Gloor, G. B. (2014). Unifying the analysis of high-throughput sequencing datasets: Characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis, *Microbiome*. BioMed Central Ltd., 2(1).

- Goncalves, L. F., Fermiano, D., Feres, M., Figueiredo, L. C., Teles, F. R., Mayer, M. P., & Faveri, M. (2012). Levels of *Selenomonas* species in generalized aggressive periodontitis. *Journal of Periodontal Research* 47, 711-8.
- Griffiths, R. I., Whiteley, A. S., O'Donnell, A. G. & Bailey, M. J. (2000). Rapid Method for Coextraction of DNA and RNA from Natural Environments for Analysis of Ribosomal DNA- and rRNA-Based Microbial Community Composition. *Applied Environmental Microbiology* 66, 5488–5491.
- Guerrero, A., Griffiths, G. S., Nibali, L., Suvan, J., Moles, D. R., Laurell, L. & Tonetti, M. S. (2005). Adjunctive benefits of systemic amoxicillin and metronidazole in non-surgical treatment of generalized aggressive periodontitis: a randomized placebo-controlled clinical trial. *Journal of Clinical Periodontology* 32, 1096-1107.
- Hagenfeld, D., Koch, R., Jünemann, S., Prior, K., Harks, I., Eickholz, P., Hoffmann, T., Kim, T.S., Kocher, T., Meyle, J., Kaner, D., Schlagenhauf, U., Ehmke, B. & Harmsen, D. (2018) Do we treat our patients or rather periodontal microbes with adjunctive antibiotics in periodontal therapy? A 16S rDNA microbial community analysis. *PLoS One* 18, 13(4):e0195534.
- Hajishengallis, G., & Lamont, R.J. (2014). Breaking bad: manipulation of the host response by *Porphyromonas gingivalis*. *European Journal of Immunology* 44, 328–338.
- Hamp, S. E., Nyman, S. & Lindhe, J. (1975). Periodontal treatment of multi rooted teeth. *Journal of Clinical Periodontology* 2, 126–135.
- Han, J., Wang, P., Ge, S. (2017) The microbial community shifts of subgingival plaque in patients with generalized aggressive periodontitis following non-surgical periodontal therapy: a pilot study. *Oncotarget* 7, 8:10609-10619.
- Hanioka, T., Morita, M., Yamamoto, T., Inagaki, K., Wang, P. L., Ito, H., Morozumi, T., Takeshita, T., Suzuki, N., Shigeishi, H., Sugiyama, M., Ohta, K., Nagao, T., Hanada, N., Ojima, M., & Ogawa, H. (2019). Smoking and periodontal microorganisms. *Japanese Dental Science Review* 55, 88-94.
- Haubek, D., Ennibi, O. K., Poulsen, K., Vaeth, M., Poulsen, S., & Kilian, M. (2008). Risk of aggressive periodontitis in adolescent carriers of the JP2 clone of *Aggregatibacter (Actinobacillus) actinomycetemcomitans* in Morocco: a prospective longitudinal cohort study. *Lancet* 371, 237-42.
- Höglund, Åberg, C., Kwamin, F., Claesson, R., Dahlén, G., Johansson, A., & Haubek, D. (2014). Progression of attachment loss is strongly associated with presence of the JP2 genotype of *Aggregatibacter actinomycetemcomitans*: a prospective cohort study of a young adolescent population. *Journal of Clinical Periodontology* 41, 232-41.

- Ikeda, E., Shiba, T., Ikeda, Y., Suda, W., Nakasato, A., Takeuchi, Y., Azuma, M., Hattori, M. & Izumi, Y. (2019) Japanese subgingival microbiota in health vs disease and their roles in predicted functions associated with periodontitis. *Odontology*. 2019 Sep 9. doi: 10.1007/s10266-019-00452-4.
- Jünemann, S., Prior, K., Szczepanowski, R., Harks, I., Ehmke, B., Goesmann, A., Stoye, J. & Harmsen, D. (2012) Bacterial community shift in treated periodontitis patients revealed by ion torrent 16S rRNA gene amplicon sequencing. *PLoS One* 7, e41606.
- Kim, Y. T., Choi, J. K., Kim, D. H., Jeong, S. N., & Lee, J. H. (2019). Association between health status and tooth loss in Korean adults: longitudinal results from the National Health Insurance Service-Health Examinee Cohort, 2002-2015. *Journal of Periodontal Implant Science*, 49, 158–170.
- Kirst ME, Li EC, Alfant B, Chi YY, Walker C, Magnusson I, Wang GP. (2015). Dysbiosis and alterations in predicted functions of the subgingival microbiome in chronic periodontitis. *Applied Environmental Microbiology* 81, 783-93
- Lafaurie, G. I., Contreras, A., Baron, A., Botero, J., Mayorga-Fayad, I., Jaramillo, A., Giraldo, A., Gonzalez, F., Mantilla, S., Botero, A., Archila, L. H., Díaz, A., Chacón, T., Castillo, D. M., Betancourt, M., Del Rosario, Aya, M., & Arce, R. (2007). Demographic, clinical, and microbial aspects of chronic and aggressive periodontitis in Colombia: a multicenter study. *Journal of Periodontology* 78, 629-39.
- Lang, N., Bartold, P.M., Cullinan, M., Jeffcoat, M., Mombelli, A., Murakami, S., Page, R., Papapanou, P., Tonetti, M., & Dyke, T.V. (1999). Consensus Report: Aggressive Periodontitis. *Annals of Periodontology* 4, 53–53.
- Laster, L., Laudenschlager, K. W. & Stoller, N. H. (1975). An evaluation of clinical tooth mobility measurements. *Journal of Periodontology* 46, 603–607.
- Ley, R.E. (2010). Obesity and the human microbiome. *Current Opinion Gastroenterology* 26, 5.
- Loe, H. (1967). The Gingival Index, the Plaque Index and the Retention Index Systems. *Journal of Periodontology* 38, 610–6.
- Lozupone, C., Lladser, M. E., Knights, D., Stombaugh, J. and Knight, R. (2011). UniFrac: an effective distance metric for microbial community comparison. *ISME Journal* 5, 169–172.
- Mandell, R. L., Ebersole, J. L., Socransky, S. S. (1987). Clinical immunologic and microbiologic features of active disease sites in juvenile periodontitis. *Journal of Clinical Periodontology* 14, 534-40.
- Morton, J. T., Sanders, J., Quinn, R. A., McDonald, D., Gonzalez, A., Vázquez-Baeza, Y., Navas-Molina, J. A., Song, S. J., Metcalf, J. L., Hyde, E. R., Lladser, M., Dorrestein, P. C. and Knight, R. (2017). Balance Trees Reveal Microbial Niche Differentiation. *mSystems* 2, e00162-16.
- Müller Campanile, V., Megally, A., Campanile, G., Gayet-Ageron, A., Giannopoulou, C., & Mombelli, A. (2019). Risk factors for recurrence of periodontal disease in patients in maintenance care in a private practice. *Journal of Clinical Periodontology* 46, 918-926.

- Nibali, L., D'Aiuto, F., Ready, D., Parkar, M., Yahaya, R., & Donos, N. (2012). No association between *A. actinomycetemcomitans* or *P. gingivalis* and chronic or aggressive periodontitis diagnosis. *Quintessence International* 43, 247-54.
- Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, Flemmig TF, Garcia R, Giannobile WV, Graziani F, Greenwell H, Herrera D, Kao RT, Kebschull M, Kinane DF, Kirkwood KL, Kocher T, Kornman KS, Kumar PS, Loos BG, Machtei E, Meng H, Mombelli A, Needleman I, Offenbacher S, Seymour GJ, Teles R & Tonetti MS (2018). Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *Journal of Periodontology* 89 Suppl 1, S173-S182
- Paster, B.J., Boches, S.K., Galvin, J.L., Ericson, R.E., Lau, C.N., Levanos, V.A., Sahasrabudhe, A., & Dewhirst, F.E. (2001). Bacterial Diversity in Human Subgingival Plaque. *Journal of Bacteriology* 183, 3770–3783.
- R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Riep, B., Edesi-Neuss, L., Claessen, F., Skarabis, H., Ehmke, B., Flemmig, T. F., Bernimoulin, J. P., Gobel, U. B., & Moter, A. (2009). Are putative periodontal pathogens reliable diagnostic markers? *Journal of Clinical Microbiology* 47, 1705-11.
- Schlafer S, Riep B, Griffen AL, Petrich A, Hubner J, Berning M, Friedmann A, Gobel UB, & Moter A. (2010). Filifactor alocis--involvement in periodontal biofilms. *BMC Microbiology* 10, 66.
- Schulz, P., Grosse, H., Schaller, H. G. & Reichert, S. (2019). Comparison of the oral microbiome of patients with generalized aggressive periodontitis and periodontitis-free subjects. *Archives of Oral Biology* 99, 169-176.
- Shaddox, L.M., Huang, H., Lin, T., Hou, W., Harrison, P.L., Aukhil, I., Walker, C.B., Klepac-Ceraj, V. & Paster, B.J. (2012) Microbiological characterization in children with aggressive periodontitis. *Journal of Dental Research* 91, 927-33
- Sousa, V., Nibali, L., Spratt, D., Dopico, J., Mardas, N., Petrie, A., & Donos, N. (2017). Peri-implant and periodontal microbiome diversity in aggressive periodontitis patients: a pilot study. *Clinical Oral Implants Research* 28, 558–570.
- Tonetti, M. S., Greenwell, H., & Kornman, K. S., (2018). Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *Journal of Periodontology* 89, S159–S172.
- Vartoukian, S.R., Palmer, R.M., & Wade, W.G. (2010). Cultivation of a *Synergistetes* strain representing a previously uncultivated lineage. *Environmental Microbiology* 12, 916–928.
- Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. New York: Springer-Verlag.

- Accepted Article
- Zambon, J. J., Christersson, L. A., & Slots, J. (1983). *Actinobacillus actinomycetemcomitans* in human periodontal disease. Prevalence in patient groups and distribution of biotypes and serotypes within families. *Journal of Periodontology* 54, 707-11.
 - Zhang, W., Luo, J., Dong, X., Zhao, S., Hao, Y., Peng, C., Shi, H., Zhou, Y., Shan, L., Sun, Q., Li, Y., & Zhao, X. (2019). Salivary Microbial Dysbiosis is Associated with Systemic Inflammatory Markers and Predicted Oral Metabolites in Non-Small Cell Lung Cancer Patients. *Journal of Cancer* 10, 1651–1662.

TABLES

Table 1. Study population characteristics*.

		<i>Frequency</i>	<i>Percentages</i>
<i>Gender</i>	Male	27	41
	Female	39	59
<i>Systemic conditions</i>	Diabetes mellitus	2	3
	Cardiovascular disease	5	8
	Overweight+Obese	38	58
	Rheumatoid arthritis	2	3
<i>Race/ethnicity</i>	White	39	59
	Asian	7	11
	Black African/Caribbean	17	26
	Other	3	5
<i>Smoking status</i>	Smoker	8	12
	Non-smoker	58	88
<i>Diagnosis</i>	Successfully treated	13	20
	LAgP	8	12
	GAgP	44	67
<i>Disease progression</i>	Fully edentulous	1	1
	Tooth loss (+)	33	50
	Tooth loss (-)	33	50
<i>Number of teeth</i>		Mean \pm SD (Range)	
	First visit	26.3 \pm 2.0 (21-28)	
	Post-APT ³	24.3 \pm 4.6 (11-28)	
	Last visit	21.9 \pm 6.5 (0-28)	

¹ IB: Interdental Brushes, ² AB: Antibiotics, ³ APT: Active Periodontal Therapy.

* Included participants for microbiome analyses.

FIGURE LEGENDS

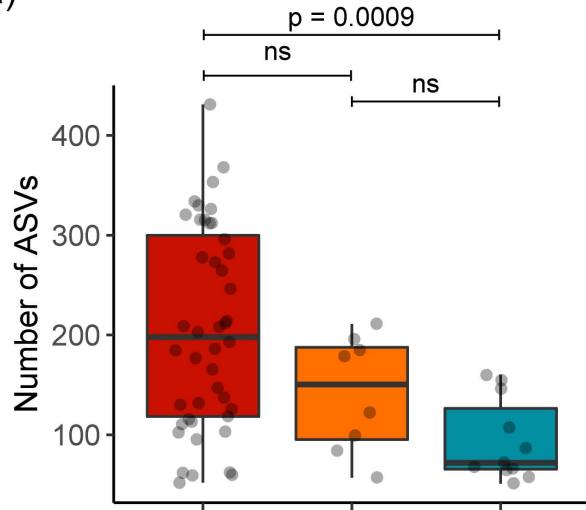
Figure 1. Alpha diversity metrics boxplot diagrams of: (a) Number of ASVs of GAgP, LAgP, and ‘successfully treated’ groups, (b) Shannon Index of GAgP, LAgP, and ‘successfully treated’ groups, (c) Faiths’s PD of GAgP, LAgP, and ‘successfully treated’ groups. Alpha diversity is significantly ($p < 0.0018$) decreased in the ‘successfully treated’ group.

Figure 2. Boxplot diagrams show the taxonomic analysis of periodontal disease (a) and health (b) - associated Genera by group: persistent GAgP (Generalised Aggressive Periodontitis), persistent LAgP (Localised Aggressive Periodontitis), and Successfully treated (stable). Statistically significant genus was highlighted in red squares.

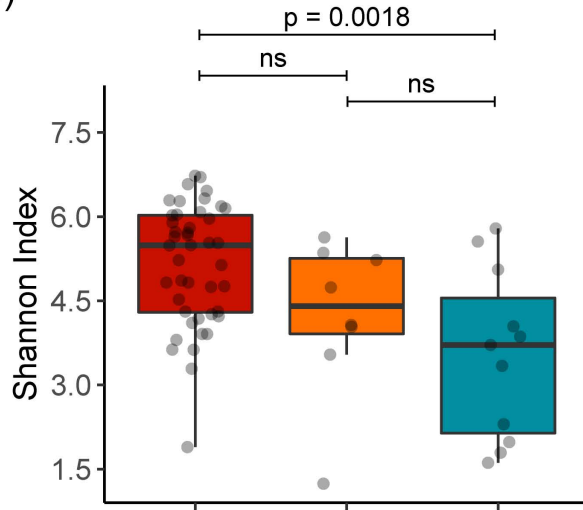
Figure 3. Boxplot diagrams show the taxonomic analysis of periodontally periodontal disease (a) and health (b) -associated species by group: persistent GAgP (Generalised Aggressive Periodontitis), persistent LAgP (Localised Aggressive Periodontitis), and Successfully treated (Stable). Statistically significant species was highlighted in red squares.

Figure 4. Beta diversity: Principal coordinate analysis (PCoA) with distance matrices (a) PCoA Aitchison, and (b) PCoA Weighted UniFrac, showing clustering of Successfully treated vs persistent GAgP vs persistent LAgP. Only GAgP vs Successfully treated resulted in significant differences. PERMANOVA of the Aitchison distances, $p = 0.004$; of the Weighted UniFrac distances, $p = 0.013$.

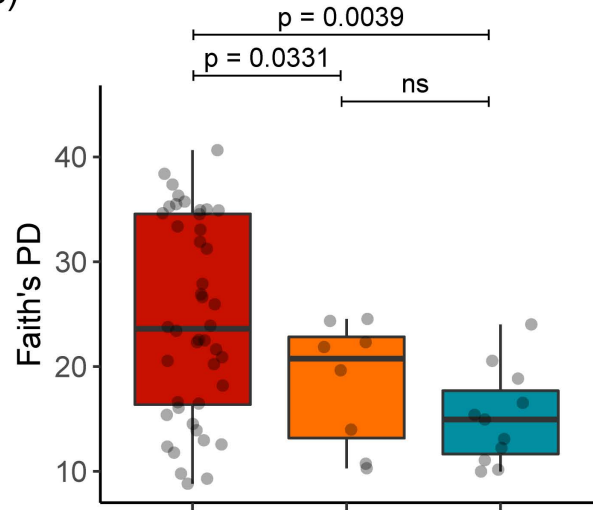
a)



b)

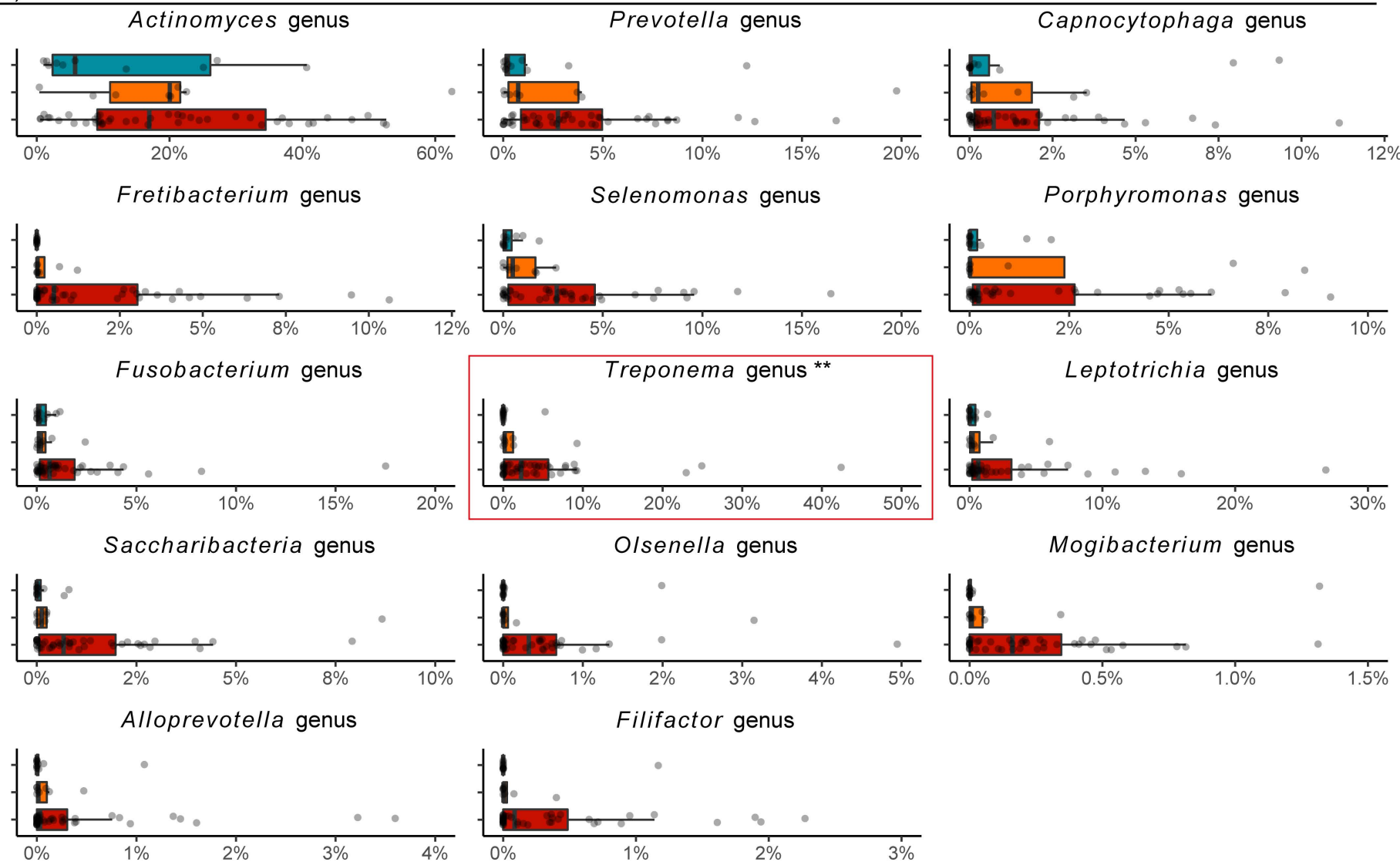


c)

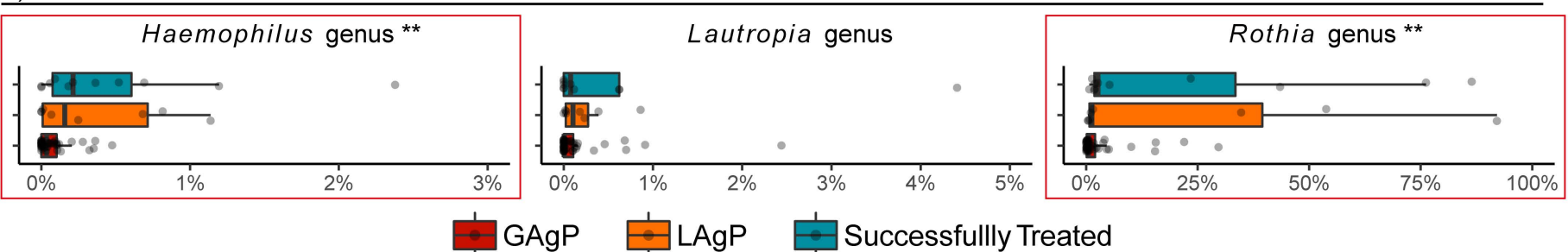


Legend: GAgP (red box), LAgP (orange box), Treated (teal box)

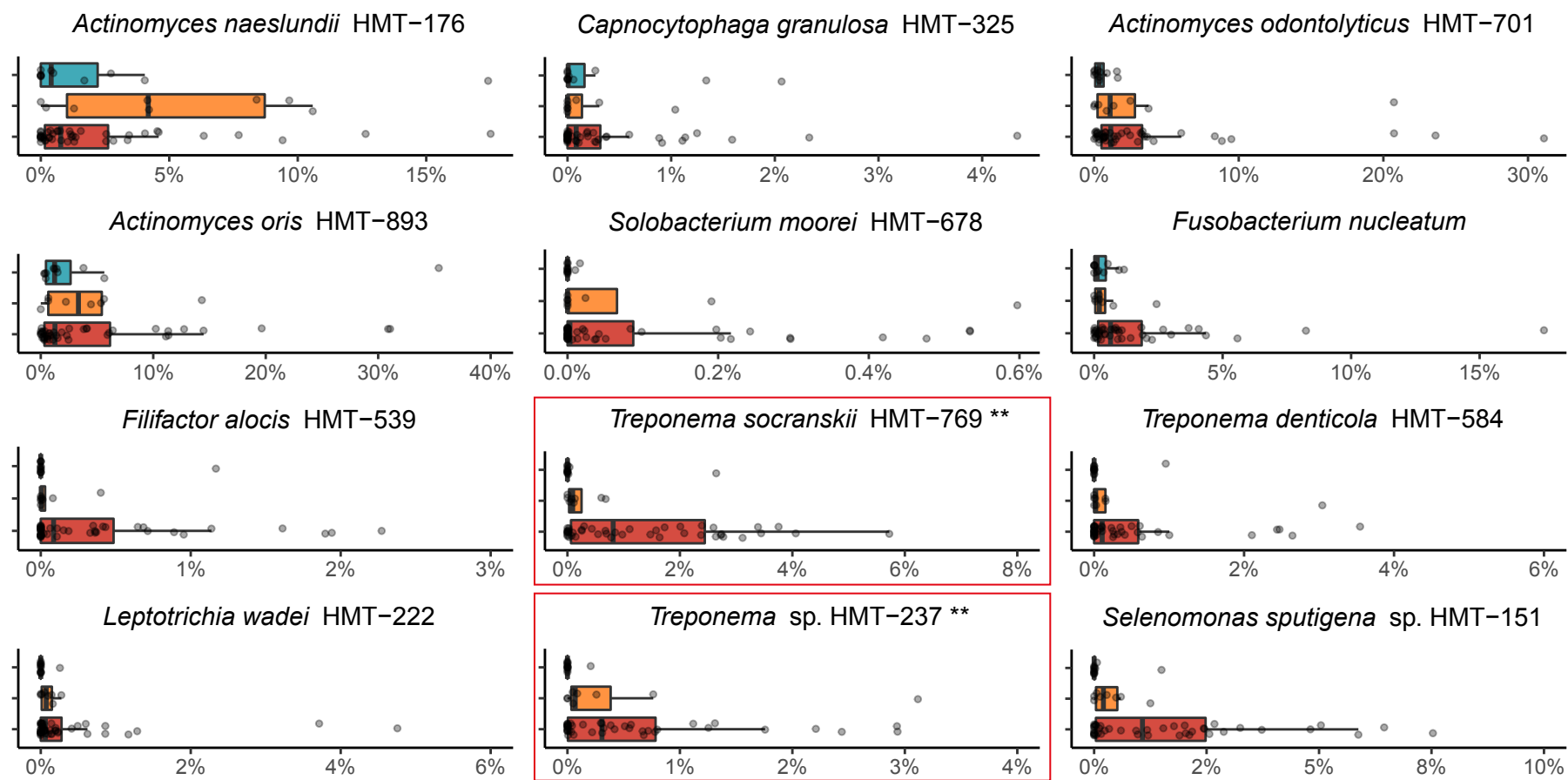
a) Perio Associated Genera



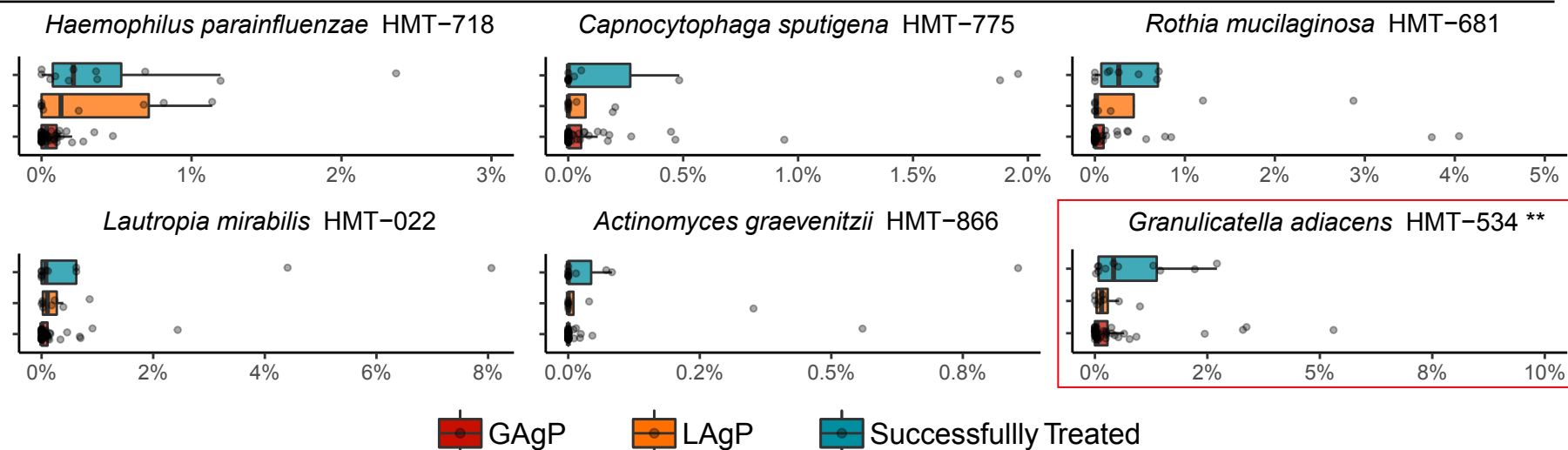
b) Health Associated Genera



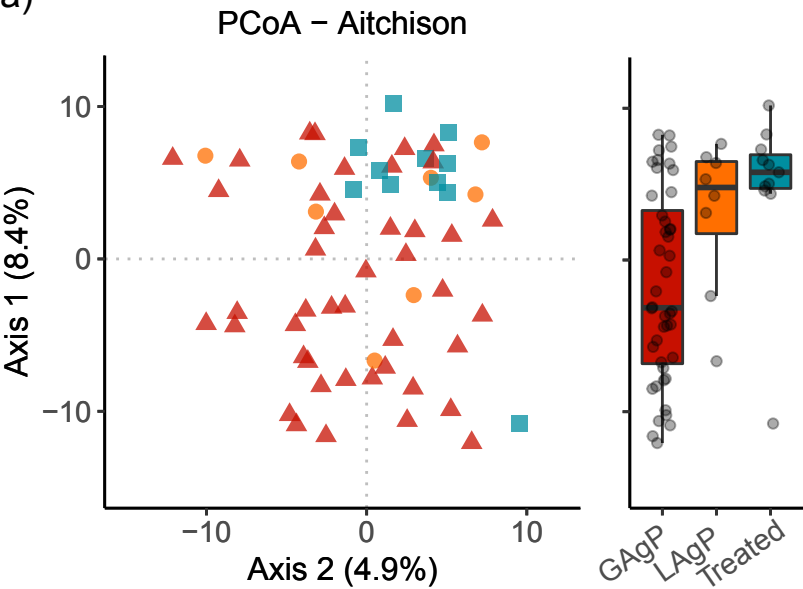
a) Perio Associated Species



b) Health Associated Species



a)



b)

